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[54]发明名称 结合态孕马混合雌激素提取方法

[57]摘要

本发明涉及一种结合态孕马混合雌激素提取方法，是利用含有氰基的极性大孔网状吸附树脂用于从孕马尿中直接或从含孕马雌激素的富集液中提取结合态孕马雌激素。该大孔网状吸附树脂是由二乙烯苯、丙烯氰等聚合而来的极性吸附树脂，具有较好的酸碱稳定性，不易结团成块，对有效成分孕马结合态雌激素吸附量高，树脂对酸碱的耐受程度高，易于再生等特点。该方法工艺简单、成本低廉、收率高、易于工业化生产。

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权利要求书

1、一种结合态孕马混合雌激素提取方法，其特征在于按下列步骤进行：

a、将孕马尿液与含有氰基的极性吸附树脂接触，动态或静态吸附均可，然后与负载有结合态混合雌激素的树脂分离；孕马尿液为不含或轻度含有粘液物质和固态物质的澄清尿液或半悬浊液；

b、用经过调节 PH 为 12.0—14.0 的水或含有 0—15%水溶性低碳醇或酮有机水溶液洗涤负载树脂；

c、将洗涤过的极性吸附树脂与能解析吸附在树脂上的结合态混合雌激素的洗脱液接触，动态或静态解析；该洗脱液为一种可与水混溶的低级醇、酮等极性有机溶剂水溶液组成，其水溶液为 PH 9—13 的碱性溶媒，解吸温度 60℃以下；

d、洗脱液经酸调节 PH 至 6—8.5 后浓缩、干燥即得结合态孕马雌激素混合物。

2、根据权利要求 1 所述的结合态孕马混合雌激素提取方法，其特征在于步骤 a 中所用的极性聚合吸附树脂是含有氰基基团的以二乙烯苯、丙烯氰等聚合而来的极性吸附树脂。

3、根据权利要求 2 所述的结合态孕马混合雌激素提取方法，其特征在于吸附树脂为大孔网状结构。

4、根据权利要求 1 所述的结合态孕马混合雌激素提取方法，其特征在于步骤 a 中一个体积的树脂吸附孕马尿的倍数为 20—100。

5、根据权利要求 1 所述的结合态孕马混合雌激素提取方法，其特征在于步骤 b 中洗脱剂为 PH 值为 12.0—14.0 的水或 15%以下的稀乙醇或丙酮溶液。

6、根据权利要求 1 所述的结合态孕马混合雌激素提取方法，其

特征在于步骤 c 中的洗脱液为一种能与水混溶的低级醇、酮等极性有机溶剂的溶液，该溶液 PH 为 9—13，其有机溶剂与水的体积比为 50：50 至 20：80。

说 明 书

结合态孕马混合雌激素提取方法

本发明涉及一种从妊娠牝马的尿液中（简称孕马尿）提取一种天然结合态混合雌激素的方法

雌激素在医学上应用于激素替代疗法，结合态混合雌激素用于治疗和预防女性生理或人工绝经期后出现的更年期综合症，能预防和治疗骨质疏松症与冠心病。天然的结合态混合雌激素以其好的疗效和耐受性得到了长久的应用。

妊娠期牝马尿中含有天然的结合态雌激素混合物，是以多种雌激素的硫酸单酯盐的形式溶解在孕马尿中，通常孕马尿每升中含有干物质 40—90 克，除尿素、马尿酸与其它常见的无机盐，有机物及粘状物外，一般结合态混合雌激素的含 以钠盐计为 80—300 毫克/升。

早期报道的结合态雌激素提取方法的专利有美国专利：US 2429398、US 2519516、US 2696265、US 2711988、US 2834712，其提取方法为先用低碳醇、酯，如正己醇、环己醇、正丁醇、乙酸乙酯、乙酸丁酯等，或低碳氯代烃如：氯仿、二氯甲烷、二氯乙烷等有机溶剂萃取，再用五至十步以上的纯化除杂得到结合态孕马雌激素。由于液—液萃取过程中出现的诸多问题，如乳化层形成、沉淀等现象，导致萃取工作困难，特别是相分离困难导致收率降低，多步骤的萃取纯化除杂也导致雌激素损失，使总收率很低。另外废弃物中溶解的萃取剂易造成环境污染。

自美国专利 US 2551205 报道活性炭吸附工艺开始，先后有 US 3769401、US 5723454、US 5814624 报道了离子交换树脂、聚丙烯酸酯吸附树脂、反相硅胶应用于结合态雌激素的提取，其中离子交换树脂由于有效组分难于从树脂中洗脱及树脂很难再生，因此应用很困

难。反相硅胶因存在再生困难和材料昂贵，导致工业成本高。中极性聚丙烯酸酯类吸附树脂虽能避免上述问题，但存在化学稳定性不高，对洗脱、再生的酸、碱强度有要求，若条件不当可导致酯基水解，另外该树脂在应用中易于结团成块，因而在应用中存在树脂吸附性能下降，导致收率降低。本发明针对目前所存在的问题，并经过大量的实验研究，研制出一种利用含有氰基的极性大孔网状吸附树脂用于从孕马尿中直接或从含孕马雌激素的富集液中提取结合态孕马雌激素的方法。

本发明目的在于，研制出的结合态孕马混合雌激素提取方法，是利用含有氰基的极性大孔网状吸附树脂用于从孕马尿中直接或从含孕马雌激素的富集液中提取结合态孕马雌激素。该大孔网状吸附树脂是由二乙烯苯、丙烯氰等聚合而来的极性吸附树脂，具有较好的酸碱稳定性，不易结团成块，对有效成分孕马结合态雌激素吸附量高，树脂对酸碱的耐受程度高，易于再生等特点。该方法工艺简单、成本低廉、收率高、易于工业化生产。

本发明所述的结合态孕马混合雌激素提取方法，按下列步骤进行：

a、将孕马尿液与含有氰基的极性吸附树脂接触，动态或静态吸附均可，然后与负载有结合态混合雌激素的树脂分离；孕马尿液为不含或轻度含有粘液物质和固态物质的澄清尿液或半悬浊液；

b、用经过调节 PH 为 12.0—14.0 的水或含有 0—15%水溶性低碳醇或酮有机水溶液洗涤负载树脂；

c、将洗涤过的极性吸附树脂与能解析吸附在树脂上的结合态混合雌激素的洗脱液接触，动态或静态解析；该洗脱液为一种可与水混溶的低级醇、酮等极性有机溶剂水溶液组成，其水溶液为 PH 9—13 的碱性溶媒，解吸温度 60℃以下；

d、洗脱液经酸调节 PH 至 6—8.5 后浓缩、干燥即得结合态孕马

雌激素混合物。

步骤 a 中所用的极性聚合吸附树脂是含有氰基基团的以二乙烯苯、丙烯氰等聚合而来的极性吸附树脂；吸附树脂为大孔网状结构；一个体积的树脂吸附孕马尿的体积倍数为 20—100。

步骤b中洗脱剂为PH值为12.0—14.0的水或含有15%以下的稀乙醇或丙酮溶液。

步骤c中的洗脱液为一种能与水混溶的低级醇、酮等极性有机溶剂的溶液，该溶液PH为9—13，其有机溶剂与水的体积比为50：50至20：80。

本发明所述的结合态孕马混合雌激素提取方法，该方法中孕马尿可用过滤或沉降的方法完全或部分除去悬浮粘状物和固体物的原尿或经浓缩的孕马尿提浓物。

在方法步骤 a 中应用含有氰基极性吸附树脂为大孔网状的有机非离子聚合物，具有亲水性结构，其表面积 300—900m^2/g，平均孔径在 40—100 埃之间。该树脂在室温下用 100 倍柱体积的孕期平均在 8 个月左右牝母的孕马尿进行静态柱吸附时，经薄层层析检测证明，未明显检出结合态雌激素泄漏。

在方法步骤 b 中洗涤负载树脂中的酚类物质洗涤液用量为树脂体积的 2—7 倍。

在方法步骤c中洗脱液为一种可与水混溶的低级醇、酮等极性有机溶剂的水溶液组成，该水溶液为无机碱溶液，如氢氧化钠或氢氧化钾，其PH值为9—13之间；洗脱液用量为树脂体积的3—8倍。

在方法步骤d中浓缩前用稀酸将洗脱液中和至6—8.5之间，酸可选择盐酸、硫酸、磷酸或醋酸；蒸发与干燥温度不超过60℃。

实施例 1

首先将50毫升含有氰基的极性大孔吸附树脂（市售，河北沧州化工厂出品的HPD—500树脂）装在柱径×柱高为2.0cm×25 cm的柱

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中，将 2 升孕马尿（不含或轻度含有粘液物质和固态物质的澄清尿液或半悬浊液）在室温下以 300 毫升/小时的流速上柱，透过液经检测，其方法为：将 2 升孕马尿的流出液最后 50 毫升用乙酸乙酯萃取后的浓缩样经薄层层析（TLC）检测，未见结合态雌激素泄漏；

然后将负载柱用经过调节 PH 为 12.0—14.0 的水或含有 0—15%水溶性低碳醇或酮稀碱液 250 毫升洗涤负载树脂，经检测，其方法为：将洗涤液中和后用同等体积的乙酸乙酯萃取后的浓缩样经薄层层析（TLC）检测，未明显见结合态雌激素洗出；

再将洗涤过的极性吸附树脂与能解析吸附在树脂上的结合态混合雌激素的洗脱液接触，静态解析；该洗脱液为一种可与水混溶的低级醇或酮等极性有机溶剂水溶液 250 毫升，PH 9—13，解吸温度 60℃以下，柱温下洗脱，先收集 25 毫升（半柱体积）为洗脱液第一段，然后再分别收集每份体积为 50 毫升的第二段、第三段、第四段、第五段；1—5 段结合态雌酮经高效液相色谱（HPLC）检测的浓度见表的 1—3 实验：

实验序号 条件	1	2	3	4
起始孕马尿结合态雌酮含量	37.5mg/L	37.5mg/L	37.5mg/L	95.2mg/L
洗涤液	PH 12.0	PH 13.0	PH 13.5	PH 14.0
洗脱液（V/V）	乙醇/水 25:75 PH 12.5	丙酮/水 50:50 PH 9.5	乙醇/水 40:60 PH 12.5	乙醇/水 35:65 PH 12.5
洗脱柱温	50℃	40℃	50℃	60℃
第一段浓度	0	0	0	0
第二段浓度	0.075mg/ml	0.543mg/ml	0.942mg/ml	2.273mg/ml
第三段浓度	0.739mg/ml	0.588mg/ml	0.255mg/ml	0.897mg/ml
第四段浓度	0.309mg/ml	0.078mg/ml	0.034mg/ml	0.104mg/ml

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第五段浓度	0.102mg/ml	0.019mg/ml	0.010mg/ml	0.035mg/ml

最后将 2—5 段的洗脱液合并，用稀酸将洗脱液中和至 6—8.5 之间（酸可选择盐酸、硫酸、磷酸或醋酸），经浓缩、干燥即得结合态孕马雌激素混合物。该混合物可直接或经进一步纯化后用于药物复配。

实施例 2

首先将 50 毫升含有氰基的极性大孔吸附树脂（市售，河北沧州化工厂出品的 HPD—500 树脂）装在柱径×柱高为 2.0cm×25 cm 的柱中，将 2 升含结合态雌酮为 95.2mg/L 的孕马尿粗提物水溶液，在室温下以 300ml/小时（5 倍柱体积/小时）的流速上柱，采用薄层层析（TLC）检测，未见结合态雌激素泄漏；

然后将负载柱用经过调节 PH 为 14.0 的稀碱水 250 毫升洗涤负载树脂，采用薄层层析检测，未见结合态雌激素明显洗出；

再将洗涤过的极性吸附树脂与能解析吸附在树脂上的结合态混合雌激素的洗脱液接触静态解析；该洗脱液为乙醇/水，其体积比为 35:65，PH 为 12.5 的水溶液 250 毫升，解吸温度 60℃，柱温下洗脱，先收集 25 毫升（半柱体积）为洗脱液第一段，然后再分别收集每份体积为 50 毫升的第二段、第三段、第四段、第五段，每段浓度见表实验 4；

最后将 2—5 段的洗脱液合并，用稀酸将洗脱液中和至 8.0（酸可选择盐酸或硫酸或磷酸或醋酸），经浓缩、干燥得结合态孕马雌激素混合物。该混合物可直接或经进一步纯化后用于药物复配。

实施例 3

首先将 50 毫升含有氰基的极性大孔吸附树脂（市售，河北沧州化工厂出品的 HPD—500 树脂）放入装有 2 升结合态雌酮为 37.5mg/L 的孕马尿中动态吸附，搅拌 30 分钟后滤出负载树脂，该树脂用 3×60ml PH 13.0 的稀碱液分次洗涤，最后负载树脂分别用 60 毫升 PH

12.5 乙醇:水的体积比为 35 :65 的稀乙醇于 50℃下分别解析三次，每次搅拌 20 分钟，滤出洗脱液，将三次洗脱液合并，经调节 PH 值于 7—8 后浓缩得出 1.043 克的结合态混合雌激素提取物，经高效液相色谱（HPLC）测试结合态雌酮含量为 6.1%，该混合物可直接或经进一步纯化后用于药物复配。

树脂再生：将待再生 50 毫升吸附树脂先用 250 毫升 PH 13.0 的 70%乙醇洗脱，再用水洗至中性，该树脂可多次重复使用。

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Patent Number : CN1308083
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Title : Extraction Method of Combined Mixed Estrin of Pregnant Mare

Abstract :

The present invention relates to a method for extracting incorporated pregnant mare mixed female sex hormone, and is characterized by utilizing cyanogroup-containing polar macroporous net-like polymeric adsorbent to directly extract the incorporated pregnant mare mixed female sex hormone from pregnant mare urine or extract it from an enrichment liquor containing pregnant mare female hormone. Said macroporous net-like polymeric adsorbent made up by polymerizing divinylbenzene and acrylic cyanogen. Said product possesses good acid and alkali stability, and it is not easy to coalesce to cake and adsorption quantity of effective component pregnant mare incorporated female hormone is high, acid-resistant and alkali-resistant extent of said polymeric adsorbent is high, and easy to be regenerated. Said method is simple in technology, low in cost, high in yield and easy to implement industrial production .

Claims :

1. A method for extracting incorporated pregnant mare mixed female sex hormone, characterized by that it is carried out according to the following steps:
   a, contacting pregnant mare urine with a cyanogroup-containing polar adsorbed resin, dynamical or static adsorption are all allowable, and then separated from the resin supported with incorporated mixed estrin; pregnant mare urine is a clear liquor or semi-suspension no containing or mild-containing viscous substance and solid substance;
   b, washing the supported resin with water of which pH value has been regulated to be 12.0 ~ 14.0 or an organic aqueous solution containing 0 ~ 15 % water-soluble low-carbon alcohol or ketone.
   c, contacting washed polar adsorbed resin with an eluting liquid capable of desorbing the incorporated mixed estrin from the resin on which the estrin has been adsorbed, dynamical or static desorption are all allowable; Said eluting liquid is an aqueous solution of polar organic solvent composed of water-soluble organic solvent such as lower alcohol, ketone etc., aqueous solution of which is a basic resolver with a pH value of 9 ~ 13, and desorption temperature less than 60 ^{0}C .
   d, regulating the pH value of the eluting liquid to 6 ~ 8.5 with acid, then concentrating , drying and just obtaining a mixture of the incorporated pregnant mare female sex hormones.

2.Said method for extracting incorporated pregnant mare mixed female sex hormone according to claim 1, characterized in that in the step a, the polar polymeric adsorbed resin used is a polar cyanogroup-containing adsorbed resin made up by polymerizing divinylbenzene, acrylic cyanogen etc.

3.Said method for extracting incorporated pregnant mare mixed female sex hormone according to claim 1, characterized in that the adsorbed resin is of a macropore net-like structure .

4.Said method for extracting incorporated pregnant mare mixed female sex hormone according to claim 1, characterized in that in the step a, the volume multiple of adsorbing pregnant mare urine by one volume of resin is 20 ~ 100 .

5. Said method for extracting incorporated pregnant mare mixed female sex hormone according to claim 1,characterized in that in the step b, the eluting liquid is water having pH value of 12.0 ~

14.0 or ethanol or acetone diluting solution having a concentration less than 15 %.

6. Said method for extracting incorporated pregnant mare mixed female sex hormone according to claim 1,characterized in that in the step c, eluting liquid is a solution of polar organic solvent which is able to mix with water such as lower alcohol, ketone, and said solution has pH value of 9 ~ 13, the ratio of organic solvent to water of it is 50 : 50 to 20 : 80.

Specfication

Extraction Method of Combined Mixed Estrin of Pregnant Mare

The present invention relates to a method for extracting natural incorporated mixed estrin from pregnant mare urine liquor (called "pregnant mare urine" for short).

Estrin is medically applied to the therapy of hormone substitution and the incorporated mixed estrin is used to cure and prevent physiological or artificial female climacteric syndrome occurred after menopause , to cure and prevent the osteoporosis and coronary heart disease. Natural incorporated mixed estrin has been applied for a long time due to its good curative effect and enduring capacity .

There is natural incorporated estrin mixture in the pregnant mare urine, which is dissolved in the pregnant mare urine in the form of mono-sulfovinate salt, generally each liter of pregnant mare urine comprising dry substance of 40 ~ 90 g, and except for urea, mare uric acid and other ordinary inorganic salt, organic substance and viscous matter, generally the content of incorporated mixed estrin is 80 ~ 300 mg /liter estimated by sodium salt .

The patents having reported the extraction method of the incorporated estrin in early stage are US 2429398, US 2519516, US 2696265, US 2711988, US 2834712, in which the extraction method is firstly extracting it with lower alcohol, such as normal hexanol, cyclohexanol , normal butanol, ethyl acetate, butyl acetate etc., or with low carbon hydrochloric ether, such as chloroform, dicholoromethane, dichloroethane and organic solvent etc., after that purifying by using removing impurity in five to more than ten steps to obtain the incorporated pregnant mare estrin. Because of various problems happened in the liquid – liquid extraction process, such as forming emulsion layer and precipitation etc., this will lead to a difficulty to carry out the extracting operation, especially, the difficulty of the phase-separation will lead to the lowering of the yield and extraction and purification by removing impurity in multiple steps will also lead to the loss of the estrin so as to reduce the yield. Besides, the extractant dissolved in the discard is easy to pollute the environment .

Beginning since the active carbon adsorption technology has been reported in American patent US 2551205, it has earlier or later been reported in US 3769401, US 5723454, US 5814624 that the ionic exchange resin , polyacrylate adsorbed resin and reversed-phase silica-gel has been applied to the extraction of the incorporated estrin, among them, the ionic exchange resin is difficulty to be regenerated due to not easy to elute the effective component from the resin so it is difficulty to be used. The reversion-phase silica-gel is not easy to be regenerated and the material is very expansive , so leading to a high industrial cost. Although medium polar polyacrylate-like adsorbed resin can avoid the problems mentioned above, the chemical stability of it is not high and there is a limit to the strength of acid and alkali used in elution and regeneration, if the improper conditions are used, it will lead to the hydrolysis of the ester group, besides, said resin is easy to be agglomerated in the course of its utilization, thus in that course, the adsorbing capacity of the resin will be reduced and so is its yield . In light of the problems existed at present and through a lot of experimental researches, the present invention has researched and developed a method for directly extracting incorporated pregnant mare estrin from pregnant mare urine by using a cyanogroup-containing polar macropore net-like adsorbed resin, or extracting from an enriched liquor containing pregnant mare estrin.

The goal of the present invention is to research and develop a method for extracting incorporated mixed estrin, which directly extracts the incorporated pregnant mare estrin from pregnant mare urine by using a cyanogroup-containing polar macropore net-like adsorbed resin, or extracts from an enriched liquor containing pregnant mare estrin. Said macropore net-like adsorbed ersin is a polar adsorbed resin made by polymerizing divinylbenzene and acrylic cyanogen and it is noted for having a better acidic and alkaline stability, not easy to be agglomerated, a high adsorptive capacity for effective component pregnant mare estrin, a good resistance to acid and alkali and easy to be regenerated etc.. Said method is of a simple technology, having a low cost and high yield, easy to bring about the industrial production.

The method for extracting incorporated pregnant mare mixed estrin mentioned above in the present invention is carried out by the following steps:

a, contacting pregnant mare urine with a cyanogroup-containing polar adsorbed resin, dynamical or static adsorption are all allowable, and then separated from the resin supported with incorporated mixed estrin; pregnant mare urine is a clear liquor or semi-suspension no containing or mild-containing viscous substance and solid substance;

b, washing the supported resin with water of which pH value has been regulated to be 12.0 ~ 14.0 or an organic aqueous solution containing 0 ~ 15 % water-soluble low-carbon alcohol or ketone.

c, contacting washed polar adsorbed resin with an eluting liquid capable of desorbing the incorporated mixed estrin from the resin on which the estrin has been adsorbed, dynamical or static desorption are all allowable; Said eluting liquid is a polar organic solvent aqueous solution composed of water-soluble organic solvent such as lower alcohol, ketone etc., aqueous solution of which is a basic resolver with a pH value of 9 ~ 13, and desorption temperature is less than 60 ^{0}C .

d, regulating the pH value of the eluting liquid to 6 ~ 8.5 with acid, then concentrating , drying and just obtaining a mixture of the incorporated pregnant mare female sex hormones.

In the step a, the polar polymeric adsorbed resin used is a cyanogroup-containing polar adsorbing resin made up by polymerizing divinylbenzene, acrylic cyanogen etc.; the adsorbed resin has a macropore net-like structure ; the volume multiple of adsorbing pregnant mare urine by one volume of resin is 20 ~ 100 .

In the step b, the eluting liquid is water having pH value of 12.0 ~ 14.0 or ethanol or acetone dilute solution having a concentration less than 15 %.

In the step c, eluting liquid is a solution of polar organic solvent which is able to mix with water such as lower alcohol, ketone, and said solution has pH value of 9 ~ 13, the ratio of organic solvent to water of it is 50 : 50 to 20 : 80.

In the method for extracting incorporated pregnant mare mixed female sex hormone said in the present invention, filtration or precipitation method can be used to remove totally or partly the suspending viscous matter and original urine of solid substance or concentrate of pregnant mare urine by concentrating.

In the step a, cyanogroup-containing polar adsorbed resin used is a macropore net-like organic nonionic polymer having a hydrophilic structure and surface area being 300 ~ 900 m^2 /g, mean pore diameter being in the range of 40 ~ 100 angstron . At room temperature the static adsorption is carried out by using 100 multiple volume of pregnant mare urine from a pregnant mare with pregnancy of mean eight months, and through determination by thin layer chromatography, it is proved that the leak of the incorporated estrin has not been detected obviously.

In the step b, during washing the supported resin, the quantity of phenol-like washing agent is 2 ~ 7 times as big as resin by volume.

In the step c, the eluctant is an aqueous solution made from water-soluble lower alcohol, ketone and organic solvent etc., which is an inorganic alkaline solution, such as sodium hydroxide or potassium hydroxide, of which pH value is in the range of 9 ~ 13; the quantity of eluctant used is 3 ~ 8 times as big as resin by volume.

In the step d, before concentration eluctant is neutralized with diluting acid to pH value in the range of 6 ~ 8, in which the acid can be selected from a group consisted of hydrochloride, sulfuric acid or acetic acid; the temperature of evaporation and drying is not more than 60 ^{0}C.

Example 1

Firstly, 50 ml of cyanogroup-containing polar macropore adsorbed resin (sale in the market, HPD-500 resin produced by Cangzhou Chemical Factory, Hebei Province) was charged into a column having column diameter × column high being 2.0 cm × 25 cm, and 2 liters of pregnant mare urine(a clear liquor or semi-suspension no containing or mild-containing viscous substance and solid substance) flowed through the column in a flow rate of 300 ml /hr at room temperature, the transmitting liquid would be detected by a method in which final 50 ml of elution liquid taken from 2 liters of elution liquid of the pregnant mare urine was extracted with ethyl acetate and after the extractive being concentrated, the sample of the concentrate was detected by thin layer chromatography (TLC), it was not found to leak the incorporated estrin;

After that, 250 ml of water of which the pH value has been regulated to 12.0 ~ 14.0 or 250 ml of diluting alkaline solution containing 0 ~ 15 % water-soluble lower carbon alcohol or ketone was used to wash the supported resin in supported column, and through detection by the way of neutralizing the washing liquid, then extracting with ethyl acetate in equal volume, and the concentrated sample of it being detected by thin layer chromatography (TCL), it was not found to wash the incorporated estrin out obviously;

Again the washed polar adsorbed resin was taken to contact with a eluting liquid capable of desorbing the incorporated mixed estrin adsorbed on the resin, by static desorption; said eluting liquid was 250 ml of a polar organic solvent aqueous solution of water-soluble lower alcohol or ketone etc., having pH value of 9 ~ 13, desorption temperature being less than 60 ^{0}C, eluting at column temperature, and first collecting 25 ml (half a column volume) of it as the first section of eluting liquid, subsequently again collecting each 50 ml of it as the second section , the third section, the fourth section and the fifth section of eluting liquid; The concentration of the incorporated estrone in the sections of 1 ~ 5 detected by high performance liquid chromatography (HPLC) can be seen experiments 1 ~ 3 in the table:

experimental number conditions	1	2	3	4
initial content of pregnanted mare urine incoporated estrinone	37.5 mg /L	37.5 mg /L	37.5 mg /L	95.2 mg /L
washing liquid	pH 12.0	pH 13.0	pH 13.5	pH 14.0
eluting liquid (V / V)	ethanol/water 25:75 pH 12.5	acetone/water 50:50 pH 9.5	ethanol/water 40:60 pH 12.5	ethanol/water 35:65 pH 12.5
temperature of eluting column	50 ^{0}C	40 ^{0}C	50 ^{0}C	60 ^{0}C
concentration in first section	0	0	0	0
concentration in second section	0.075 mg /ml	0.543 mg /ml	0.942 mg /ml	2.273 mg /ml
concentration in third section	0.739 mg /ml	0.588 mg /ml	0.255 mg /ml	0.897 mg /ml
concentration in fourth section	0.309 mg /ml	0.078 mg /ml	0.034 mg /ml	0.104 mg /ml
concentration in fifth section	0.102 mg /ml	00192 mg /ml	0.010 mg /ml	0.035 mg /ml

Finally, the eluting liquid from 2 ~ 5 sections were merged into one and neutralized with diluting acid to pH value being in the range of 6 ~ 8.5(the acid can be selected from a group consisted of hydrochloric acid, sulfuric acid, phosphoric acid or acetic acid), after concentrated, dried, an incorporated pregnant mare estrin mixture could be obtained. Said mixture is able directly or through further purifying to be used to compound medicine.

Example 2

Firstly, 50 ml of cyanogroup-containing polar macropore adsorbed resin (sale in the market, HPD-500 resin produced by Cangzhou Chemical Factory, Hebei Province) was charged into a column having column diameter × column high being 2.0 cm × 25 cm, and 2 liters of aqueous solution of rough extracting substance of pregnant mare urine containing incorporated estrinone of 95.2 mg /L, flowed through the column in a flow rate of 300 ml /hr (5 times of column volume/ hr.) at room temperature, the sample of the concentrate was detected by thin layer chromatography (TLC), it was not found to leak the incorporated estrin;

After that, 250 ml of diluting alkaline water of which the pH value has been regulated to 14.0 was used to wash the supported resin in supported column, and detected by thin layer chromatography (TCL), it was not found to wash the incorporated estrin out obviously;

Again the washed polar adsorbed resin was taken to contact with a eluting liquid capable of desorbing the incorporated mixed estrin adsorbed on the resin, by static desorption; said eluting liquid was 250 ml of an aqueous solution in which the volume ratio of alcohol to water was 35 : 65, pH value was 12.5, desorption temperature was 60 ^{0}C, eluting at column temperature, and first collecting 25 ml (half a column volume) of it as the first section of eluting liquid, subsequently again collecting each 50 ml of it as the second section , the third section, the fourth section and the fifth section of eluting liquid; The concentration of each section can be seen experiments 4 in the table;

Finally, the eluting liquid from 2 ~ 5 sections were merged into one and neutralized with diluting acid to pH value being 8 (the acid can be selected from a group consisted of hydrochloric acid, sulfuric acid, phosphoric acid or acetic acid), after concentrated, dried, an incorporated pregnant mare estrin mixture could be obtained. Said mixture is able directly or through further purifying to be used to compound medicine.

Example 3

Firstly, 50 ml of cyanogroup-containing polar macropore adsorbed resin (sale in the market, HPD-500 resin produced by Cangzhou Chemical Factory, Hebei Province) was charged into 2 liters of pregnant mare urine containing incorporated estrinone of 37.5 mg /L to carried out dynamical adsorption , stirring for 30 minutes, then the supported resin was filtered out , said resin was washed separately with 3 x 60 ml of diluting alkaline liquid having pH value of 13.0, finally the supported resin was desorbed three times with 60 ml of diluting ethanol in which the volume ratio of ethanol to water was 35 : 65, stirring for 20 minutes per time, filtering eluting liquid out, merging eluting liquid in three times together , through regulating pH value to 7 ~ 8, then concentrated to obtain the extracting substance of the incorporated mixed estrin of 1.043 g , the content of the incorporated estrinone being 6.1 % determined by high performance liquid chromatography (HPLC). Said mixture is able directly or through further purifying to be used to compound medicine.

Regeneration of resin: 50 ml pre-regenerating adsorbed resin was eluted with 250 ml of 70 % ethanol having pH value of 13.0, and washed again with water to neutral , the resin can be utilized repeatedly many times.